III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health effects Division/Office of Pesticides Program/U.S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable (Acceptable/Guideline)

This study is fully compliant with OECD 424 (1997)

C. CONCLUSIONS

The no-observed-adverse-effect-level (NOAEL) for systemic effects was determined as 160 mg/kg/day based on the occurrence of reduced weight gain in males at the LOAEL of 640 mg/kg/day. It was concluded that penthiopyrad did not elicit functional or morphological evidence of neurotoxicity at target dose levels of up to the maximum tolerated dose of 640 mg/kg/day. Therefore, a NOAEL for neurotoxicity was established as 640 mg/kg/day.

IIA 5.7.5 Postnatal developmental neurotoxicity

Report:	IIA 5.7.5/01 Stannard, D. R. (2009a): MTF-753 - Preliminary developmental neurotoxicity study by oral gavage administration to CD rats; Huntingdon Life Sciences Ltd. Cambridgeshire PE28 4HS, England; unpublished report no. MTU/0417; March 31, 2009IIA , MRID 47614916
Report:	5.7.5/02 Stannard, D. R. (2009b): MTF-753: Developmental neurotoxicity study in the CD rat by oral (gavage) administration; Huntingdon Life Sciences Ltd. Cambridgeshire PE28 4HS, England; unpublished report no. MTU0419; July 14, 2009, MRID 47614917
Dates of work:	July 31, 2008 - March 09, 2009 (main study)
Guidelines:	OCSPP 870.6300 (1998) OECD Guideline 426 (2007)
GLP:	Yes (certified laboratory) for the main study.
GLP Exemptions	The preliminary study generally conformed to good laboratory practice principles, but was not performed according to GLP standards published by US-EPA

Executive summary:

In a developmental neurotoxicity study (MRID 47614917), three groups of 22 mated Crl:CD (SD) IGS BR female rats received penthiopyrad (purity 99.2% w/w; Lot No. 2000111) by gavage at doses of 0, 100, 250 or 500 mg/kg/day from gestation day 6 to lactation day 6, inclusive, and their offspring were dosed from postnatal day 7 to postnatal day 20 or 21, inclusive. A similar control group received the vehicle only for the same period. A functional operation battery (FOB) of behavioral assessments was performed periodically on both the parent females (12/group) and their offspring (up to 22/sex/group). Quantitative assessment of motor activity, sensory function and learning and memory were also performed on the offspring. Groups of 10 male and 10 female offspring/group were perfused on days 21 or 66 of age for detailed neuropathological evaluation. Assessments of clinical condition, bodyweight performance, food consumption, gestation length and parturition observations were also conducted on the parental females. Clinical condition, litter size and survival, sex ratio, bodyweight and sexual maturation were also assessed for the litters/offspring.

Oral treatment of pregnant and lactating female rats with penthiopyrad was well tolerated and there were no treatment-related adverse findings, behavioural effects or reproductive effects at any dose level. At 500 or 250 mg/kg/day, mean offspring bodyweights on day 1 of age were marginally lower than control, and subsequent bodyweight gain to Day 4 of age was low in both sexes at 500 mg/kg/day and in males at 250 mg/kg/day. Following the start of offspring treatment on Day 7 of age, mean bodyweight gain of both sexes at 500 or 250 mg/kg/day groups was lower than control to scheduled termination. In addition, signs of perianal staining were observed for many offspring receiving 500 or 250 mg/kg/day.

Effects on the FOB were confined to offspring receiving 500 mg/kg/day, in which a treatment-related increased incidence of occasional slight whole body tremors was apparent at Day 21 of age, but not subsequently after discontinuation of treatment. Motor activity (rearing and cage floor activity) for males and females at 500 mg/kg/day was consistently high relative to the controls on all test days. Motor activity scores for females receiving 250 mg/kg/day and for males and females receiving 100 mg/kg/day were unaffected by treatment at all testing intervals. There was no effect of treatment on auditory startle response pre-pulse inhibition, but peak startle amplitude values with and without a pre-pulse in female offspring at 500 mg/kg/day group were significantly lower than the controls at day 61/62 of age. There was no effect of treatment at any dose level on the learning and memory capacity of the offspring as assessed by swimming maze. Sexual maturation, assessed by the time of vaginal opening or balano-preputial separation was unaffected by treatment at all dose levels.

There were no treatment-related macroscopic findings in the offspring at scheduled termination, no effects on brain weights, no histopathological changes in the tissues of the central and peripheral nervous systems presented for neuropathological examination on day 21 or day 66 of age and no changes in brain morphometry on day 21 or day 66 of age.

It was concluded that the maternal no-observed-adverse-effect-levels (NOAEL) was 500 mg/kg/day, based on the absence of maternal adverse effects up to the highest dose tested. The NOAEL for the F1 offspring was 100 mg/kg/day based on decreased body weights up to PND 7 observed at 250 mg/kg/day.

This study is classified as totally reliable (acceptable/non-guideline) and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OCSPP 870.6300; OECD 426 (draft)) due to the pending review of the positive control data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: MTF-753 Penthiopyrad, technical

Description: White powder

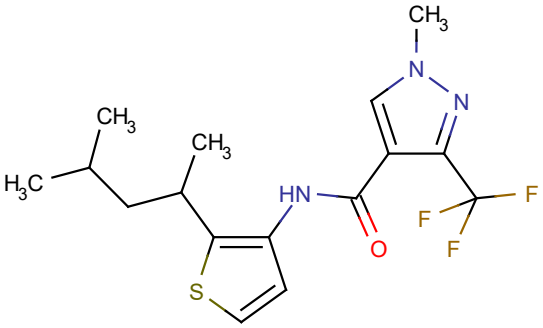
Lot/Batch#: 2000111

Purity: 99.2% w/w

CAS #: 183675-82-3

Stability of test compound: Stable under storage conditions until at least April 30, 2011

Structure:



2. Vehicle and/or positive control: Aqueous carboxymethylcellulose 0.5% / Tween 80, 0.1% w/v

3. Test animals

Species: Rat

Strain: Crl:CD (SD) IGS BR

Age: 10 - 11 weeks

Weight at dosing: 236 - 283 g

Source: Charles River (UK) Ltd, England

Acclimation period: At least 5 days

Diet: SDS VRF1 certified diet (Special Diet Services) *ad libitum* except during neurobehavioral evaluations

Water: Local potable tap water *ad libitum*

Housing: 1:1 during mating; 1/cage during gestation; dam + litter during lactation; up to 4 F1/cage post-lactation

Husbandry: Depending on the study phase, either stainless steel grid cages suspended above absorbent paper or solid floor polycarbonate cages with wood shaving bedding (Lignocel type 3-4 wood flakes)

No. of animals used per dose/time point: 22 mated females

4. Environmental conditions:

Temperature: 21 ± 2°C

Humidity:	40 - 70%		
Air changes:	Not specified, but non-re-circulated air.		
Photoperiod:	12 hours light/12 hours darkness		
5. Test compound administration:	**Dose levels**	**Final Volume**	**Route**
Main Study	0, 100, 250 or 500 mg/kg/day	5 mL/kg	Gavage

B. STUDY DESIGN AND METHODS

1. In life dates: 11 August, 2008 – 22 November, 2008

2. Animal assignment and treatment: The females were mated on a 1:1 basis (16-day mating period) with stock males of the same strain and source and assigned to treatment groups on day 0 of gestation (day 0 of gestation = positive vaginal smear or ejected copulation plugs present). Similar numbers of mated females were allocated to each treatment group on each day of mating and each male inseminated only one female/treatment group.

3. Dose selection: The dose levels used were selected based on the results of a preliminary study (Huntingdon Life Sciences Report Number: MTU0417, IIA 5.7.5/01), in which 4 groups of 8 maternal animals, and their pups, were directly treated by gavage at dose levels of 0, 100, 300 or 1000 mg/kg/day, from gestation day 6 to lactation day 7, and from day 7 to day 20/21 of age for offspring. During the study, clinical condition, bodyweight, food consumption, gestation length and parturition observations, macroscopic pathology and brain weight investigations were performed on the adult females. Clinical condition, litter size and offspring survival, sex ratio, bodyweight, macroscopic pathology and brain weight investigations were undertaken on the litters/offspring. Motor activity was also assessed on selected offspring on Day 22.

Treatment of the maternal animals at doses up to 1000 mg/kg/day was well tolerated throughout the treatment period in the preliminary study, but pup weight loss, deterioration in clinical condition and offspring death occurred at 1000 mg/kg/day, and slight, transiently decreased pup weight gain occurred at 300 mg/kg/day but without effect on viability. Therefore, a high dose level of 500 mg/kg/day was selected following consultation with US-EPA and Canadian PMRA, and agreed by these regulatory bodies. The intermediate and low dose levels were set at 250 and 100 mg/kg/day, respectively, in order to delineate a dose-response relationship in the event of a treatment-related effect.

4. Dosage preparation and analysis: All formulations were prepared weekly (one bottle/dose level/day) and stored refrigerated (4^{o}C) until use. For each concentration, an appropriate amount of penthiopyrad and vehicle were weighed, ground in a pestle and mortar, and a small amount of the vehicle was added to the mortar. The mixture was then re-ground with additional vehicle to produce a smooth, pourable suspension, which was placed into an ultrasonic bath to remove aeration. The suspension was quantitatively transferred to a measuring cylinder, made up to volume with vehicle, and mixed in a high-shear homogeniser to provide a homogenous suspension. This suspension was maintained on a magnetic stirrer during sampling.

The homogeneity and stability of penthiopyrad in the vehicle was confirmed prior to the commencement of treatment as part of another study (MTF-753: Validation of an analytical method to verify the homogeneity and stability of a liquid formulation preparation, Huntingdon Life Sciences Study No. LDA 070/052445 – dossier point IIA 5.6.10/03). Satisfactory homogeneity and stability were demonstrated for 2 days at ambient storage and for 15 days under refrigerated storage at approximately 4°C.

Samples of each formulation prepared for administration on the first day of treatment during gestation and during the second week of lactation, including the vehicle control, were analysed in duplicate for achieved concentration. The mean concentrations of penthiopyrad in all test formulations analysed were within the range -5% to +2% of nominal concentrations, confirming the accuracy of formulated preparations. Penthiopyrad was not detected in the Control formulations.

5. Dose administration: Maternal animals were dosed once daily by gavage with 5 mL/kg of the aqueous suspensions from day 6 of gestation to day 6 of lactation, inclusive, but not on the day of parturition if birth was in progress at the time of dosing. Offspring were similarly dosed from day 7 to day 20 or 21 of age. The individual volumes administered were calculated from the most recent body weight.

Group	Maximum number of offspring allocated for assessment of (M/F):					
	Motor activity (set 1)	FOB tests (set 1)	Auditory startle response pre-pulse inhibition (set 2)	Learning & memory at: Day 23/24 (set 1)	Learning & memory at: Day 61 (set 3)	Brain examination on Day 21 (set 4)
3	21/21	21/21	21/21	21/21	20/21	10/10
2	20/20	20/20F	20/20	20/20	20/19	10/10
4	22/22	22/22	21/22	21/22	21/22	11/9
1	22/22	22/22	22/22	22/22	22/22	11/11

C. OBSERVATIONS:

Maternal animals: The animals were inspected at least twice a day for signs of a reaction to treatment and a full physical examination was performed on days 0, 5, 11, 17 and 20 of gestation and on days 1, 7, 14 and 21 of lactation. Twelve females per group were subjected to a detailed semi-quantitative observation battery of in-the-hand and standard arena observations (FOB), on days 12 and 18 of gestation and days 4 and 20 of lactation. The observations included ease of removal from cage, autonomic functions, fur condition, reactivity to handling, palpebral closure, posture, gait, tremors, twitches, convulsions, activity, rearing behaviour, grooming, urination and defecation. Maternal body weights and food consumption were recorded at 3 or 4-day intervals throughout gestation and lactation. The animals were observed 3 times daily for evidence of parturition from day 20 of gestation, and the duration of gestation was recorded. All females were allowed to deliver their young and rear them to day 21 of lactation.

Offspring

Litter Observations: All F1 progeny were examined *ca.* 24 hours after birth and numbers of live and dead progeny, body weights, sexes and clinical signs recorded. Subsequently, all litters were examined for clinical signs at least once daily during lactation until day 21 and then in more detail on 21, 35, 42, 49, 56, and 63 days of age. Litters were culled, by a process of random selection, to 4 progeny of each sex, where possible, on day 4 of lactation. The sexes of the progeny were re-checked on day 4 (before and after culling) and day 21. Body weights were recorded on days 4, 7 - 21 and 28 *post partum*. Offspring selected for further study were given a full physical examination weekly until termination. Unallocated progeny were retained until termination. Sexual maturation was assessed by recording the completion of balano-preputial separation or the occurrence of vaginal opening. Body weight was also recorded at these times.

For each behavioral investigation, one male and one female per litter were selected to achieve equal numbers of each sex from each group. Up to 22 male and 22 female offspring were randomly selected to give at least 19/sex/group on each investigation.

In addition, up to 11 males and 11 females/group from set 1 were selected for sacrifice and perfusion-fixation on Day 66 of age, for further brain morphometry and histopathological examination of central and peripheral nervous tissues.

The FOB was performed on days 4, 11, 21, 35, 45 and 60. On day 4, the speed of the surface righting reflex was determined semi-quantitatively, and animals were evaluated individually in a perspex arena for one minute for activity, maximum distance travelled and maximum pivoting angle. Any other abnormal locomotor or behavioral observations were also recorded. On day 11, ambulatory, rearing and grooming activity was measured and the presence or absence of urination noted. Abnormalities in coordination, physical condition and behaviour were also noted. On days 21, 35, 45 and 60, a detailed series of in-the-hand and standard arena observations was performed. Where applicable, a semi-quantitative grading system was used.

Quantitative motor activity was measured at 13, 17, 22 and 59 days of age using an automated monitoring system measuring ambulatory and rearing activity. Data were collected during 10 successive 6-minute intervals. Activity scores were produced by the interruption of light beams. Beam detectors located near the floor monitored ambulatory movement while higher beam detectors monitored rearing activity.

Sensory function was assessed by pre-pulse inhibition of the startle response on Day 23/24 and Day 61/62 of age. Where possible, the same offspring from each litter were used for both testing occasions. The animals were tested in an automated system in which individual animals were placed into a soundproof chamber and allowed an acclimatisation period of 15 minutes. Mean auditory startle amplitudes and latencies to peak response were recorded during a randomised sequence of 40 trials.

Learning & memory at 23/24 and from 61/62 days of age were evaluated in 2 separate sets of animals in a Morris water-filled swimming maze with visual cues using a series of 3 trials on each of 4 successive days for each animal. A 90-sec period was allowed to complete the task, and the time (latency) to reach a submerged and non-visible fixed platform was recorded, together with the number of pool sectors crossed. A different starting point was used for each trial. Failure to complete the task was recorded as 90 sec latency. The pattern of mean trial times, pool sector entries and failed trials on successive days was used to assess both learning (acquisition) and memory (consolidation).

4. Necropsy: For each group, excess offspring from each litter culled at day 4 of age were subjected to a full macroscopic examination if externally abnormal. Culled pups of normal appearance were discarded. All other offspring not selected for further procedures were sacrificed on day 28 and subjected to a full macroscopic examination. Offspring dying before weaning were subjected to a full macroscopic examination including assessments of milk in the stomach and evidence of mis-dosing. F0 females were sacrificed at day 21 of lactation and subjected to a full macroscopic examination including a uterine implantation site count.

Following weaning on day 21 of age, for each group, either one male or one female was selected, where possible, from each litter for perfusion-fixation and neuropathology necropsy procedures. They were sacrificed, perfusion-fixed and subjected to necropsy and post mortem examination. The brains were transected from the spinal cord above the first cervical spinal nerve, weighed, and then preserved together with any grossly abnormal tissues. Brain length and width measurements were not recorded, in error. The preserved tissues of progeny from maternal females treated at 0 or 500 mg/kg/day were processed and examined by light microscopy. The areas examined were the olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, cerebrum, tectum, tegmentum, medulla oblongata, cerebellum, and pons. In addition, morphometric measurement of the thicknesses of the neocortex, corpus callosum, hippocampus and the folia of the cerebellum (pyramis) was performed.

All day 66 F1 offspring for neuropathology were sacrificed, perfusion-fixed and subjected to necropsy and post mortem examination. The brains were transected from the spinal cord above the first cervical spinal nerve, weighed, measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum, and at the widest part of the cerebral hemispheres and then preserved. Dorsal root fibres and ganglia (cervical and lumbar), eyes, optic and sciatic nerves, skeletal muscle, spinal cord, tibial nerves (at knee joint and calf muscle branch) and ventral root fibres (cervical and lumbar), together with any grossly abnormal tissues and the carcass, were also preserved in appropriate fixative. The preserved tissues from the groups treated at 0 or 500 mg/kg/day were processed in paraffin wax or resin (sciatic and tibial nerves) and examined by light microscopy. The areas of brain examined and brain morphometric measurements were as for day 21 progeny. Spinal cord (transverse and longitudinal sections at the cervical and lumbar swellings), dorsal and ventral root fibres (longitudinal sections at the cervical and lumbar levels), dorsal root ganglia (cervical and lumbar levels), eyes, optic nerve, skeletal muscle (transverse section), and the sciatic and tibial nerves (longitudinal and transverse sections) were also examined.

In addition, the brains of all day 21 and 66 perfusion-fixed F1 animals in the low and intermediate dose groups were similarly processed to slide stage, but were not subjected to histopathological examination or brain morphometry measurements because there was no effect of treatment at the highest dose level.

In-life observations, weighings, necropsy examinations and histopathological examination and measurement procedures on maternal animals and progeny were performed without knowledge of the treatment group.

5. Statistics: Reproductive indices were calculated for gestation, post-implantation survival, live births, viability and lactation. Statistical analyses were performed, where appropriate, using frequency analysis if 75% of data across all groups were the same value. Treatment groups were compared by pairwise Fisher's exact tests for each group against the control. If Bartlett's test for homogeneity of variance was not significant at 1%, then parametric analysis was applied. A *t* test was used to compare Group 3 with Group 1 for brain morphometry. In all other instances, the F1 approximate test was applied. If the F1 approximate test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F1 approximate test was significant, suggesting that the dose response was not monotone Dunnett's test was performed.

If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. Wilcoxons's test was used to compare Group 3 with Group 1 for brain morphometry. For the comparison of Group 3 with Groups 2, 4 and 1 the following tests were performed. The H1 approximate test, the non-parametric equivalent of the F1 test described above, was applied. If the H1 approximate test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H1 approximate test was significant, suggesting that the dose-response was not monotone, Steel's test was performed.

Sex ratios were analysed by generalised mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test.
For brain weight data and startle amplitudes (Day 23/24 only), analysis of covariance was initially performed using bodyweight (terminal or most recently recorded weight, as appropriate) as covariate. If the within group relationship between organ weight/startle amplitude and bodyweight was significant at the 10% level, then the above treatment comparisons were made on adjusted group means in order to allow for the influence of bodyweight on the parameter. For Morris maze data, the reciprocal of the mean of three trials was analysed, for sector count the square route of the mean of three trials was analysed and for number of fails frequency analysis or non-parametric analysis was employed. For categorical data, such as pathological findings, the proportion of animals was analysed using Fisher's Exact test for each treated group versus the control.

6. Deficiencies: Brain length and width in day 21 offspring for neuropathology were not recorded, in error.

II. RESULTS AND DISCUSSION

Maternal animals:

There were no treatment-related premature deaths at any dose level. However, one female at 500 mg/kg/day was sacrificed on day 18 of gestation due to deteriorating clinical condition. Necropsy revealed a detached placenta and a large amount of uterine clotted blood. Since the premature death was considered not to be related to treatment, the animal was replaced. Three further animals were sacrificed prematurely, all showing signs of dystocia. A control animal was sacrificed on day 22 of gestation and 2 females at 100 mg/kg/day were sacrificed on day 20 of gestation. All animals showed distension of the vagina and uterus with serous fluid. There were no treatment-related clinical signs and body weights during gestation and lactation were unaffected by treatment at all dose levels. Food consumption during gestation was unaffected by treatment at all dose levels, but during lactation, food consumption was reduced at 250 or 500 mg/kg/day (Table 123).

Table 123
Maternal group mean food consumption during lactation

Treatment (mg/kg/day)	Group mean food consumption (g/day) on lactation days (mean±SD):					
	1 - 3	**4 - 6**	**7 - 10**	**11 - 13**	**14 - 16**	**17 - 20**
0	44±3.7	51±4.5	63±6.7	74±6.7	81±17.2	79±9.1
100	43±4.9	51±4.9	62±5.3	74±6.9	79±6.9	79±6.4
250	42±6.9	47±4.6* (↓8)[a]	55±4.5** (↓12)	67±5.9** (↓9)	72±4.8** (↓11)	74±6.4
500	41±4.6* (↓7)	48±4.0* (↓6)	52±5.4** (↓17)	66±6.3** (↓11)	73±7.5** (↓10)	75±8.2

* $p < 0.05$; ** $p < 0.01$. [a]Percent change compared to control group. Mean data found in Table 6 on pg. 94 of main study. Individual data found in Appendix 5 on pages 210-213. 71 ± 22.1 was originally reported in table for 500 mg/kg/day group on days 11-13. However, the original listed 165 g/day for animal #7. The reviewer assumes 165 g/day is a typo and the actual value should be 65 g/day. The current average for the 500 mg/kg/day group for days 11-13, 66±6.3, reflects this change. An ANOVA of revised days 11-13 compared to control indicates $p<0.05$.

Treatment of parental females with penthiopyrad at doses up to 500 mg/kg/day was well tolerated throughout the treatment period with no treatment-related clinical signs at any dose level. There was no evidence of an adverse

effect of treatment at any dose level on maternal bodyweight gain during gestation. However, there was a treatment-related decrease in food consumption in the 250 and 500 mg/kg/day dose groups during lactation. This decrease in food consumption was not considered adverse since the mean bodyweights for all treatment groups were not

significantly different (p>0.05) from Control values, with one exception. The overall mean bodyweight gain at 500 mg/kg/day during Days 1-14 of lactation (37±9.3g) was marginally lower than Control (44±12.5g) (p<0.05). However, the pattern of bodyweight change during lactation reflected the normal variation expected in dams, and was not considered a treatment-related effect.

There was no effect of treatment at any dose level on the FOB of in-the-hand and arena observations performed on gestation days 12 and 18 and lactation days 4 and 20. All minor inter-group variations were not consistent between testing intervals and were, therefore, attributable to natural variation.

All animals in the treated and control groups were pregnant and there was no effect of treatment on the duration of gestation. With the exception of the three females with dystocia, all females in all groups had a gestation length within the expected range of 22-23 days and the group mean gestation index was not affected by treatment. Similarly, there were no treatment-related differences in the post-implantation survival index, live birth index and viability index (Table 124)

There were no treatment-related macropathology findings, and absolute brain weights of maternal animals were not affected by treatment at any dose level.

Table 124
Summary of litter data and survival to day 4

Parameter	Value in group treated at (mg/kg/day):			
	0	**100**	**250**	**500**
No. mated / no. Pregnant	22 / 22	22 / 22	22 / 22	22 / 22
No. with live litters	21[a]	20 [b]	22	22
No. with total litter loss	0	0	0	0
No. with weaned progeny	21	20	22	22
Mean no. Implantations (±SD)	16.7±1.8	17.0±1.7	16.5±2.1	16.0±1.8
Mean total litter size on day 1 ±SD)	15.3±2.5	16.1±2.3	15.9±1.7	14.8±2.0
Mean live litter size on day (mean±SD):				
1	15.0±2.5	15.8±2.3	15.9±1.7	14.5±2.2
4 (pre-cull)	14.9±2.6	15.5±2.4	15.5±1.6	14.5±2.2
4 (post-cull)	8.0±0.2	8.0±0.0	8.0±0.0	8.0±0.0
[c]Gestation index (%)	95	91	100	100
[d]Post-implantation survival index (%)	91.7	94.3	96.7	92.9
[e]Live birth index (%)	97.9	98.2	100	98.0
[f]Viability index (%)	99.3	98.0	98.1	100
Sex ratio (% males) on day (mean±SD):				
1	43.8±12.8	54.1±16.3*	47.7±14.5	53.0±*14.1
4 (pre-cull)	44.0±12.7	54.1±16.2*	48.4±14.4	53.0±14.1
4 (post-cull)	49.1±4.2	53.1±9.0	50.0±0.0	50.0±3.9

* $p < 0.05$; [a] one animal with dystocia sacrificed prematurely; [b] two animals with dystocia sacrificed prematurely; [c]Gestation index = (No. live litters born/No. pregnant) x 100; [d]Post-implantation survival index = (No. offspring born/No. uterine implantation sites) x 100; [e]Live birth index = (No. offspring live on PND1/No. offspring born) x 100; [f]Viability index = (No. live offspring on PND 4 pre-culling/No. live offspring on PND1) x 100; index values defined on page 51 of main study, MRID 47614916. Group mean data found on page 114-117 of main study, MRID 47614916.

F1 offspring:

There was no effect of treatment at any dose level on the mean number of implantations, post-implantation survival index, litter size at birth, sex ratio or offspring survival up to the commencement of direct dosing on Day 7 of age. Nine neonates from 5 litters, 6 from 3 litters and 5 from 4 litters died or were sacrificed before the start of direct treatment on day 7 in the groups treated at 100, 250 or 500 mg/kg/day, respectively. These incidences were comparable to the control incidence of 8 neonates from 6 litters. The predominant macroscopic finding was absence of milk in the stomach.

The mean offspring bodyweight of both sexes at 250 or 500 mg/kg/day on Day 1 of age was marginally lower (4-6%) than the control group (Table 125). There was no clear dose response apparent, but the differences for female offspring attained statistical significance ($p < 0.05$). In addition, mean bodyweight gain for males at 250 and

both sexes at 500 mg/kg/day during Days 1-4 of age was statistically significantly lower than the controls by up to 16.1%. The mean bodyweight on Day 1 and the mean weight gain from Day 1 to Day 4 of offspring in the 100 mg/kg/day group were not significantly different from control values.

At Day 7 of age, the mean bodyweight of males and females in all treated groups was statistically significantly lower than Control ($p < 0.01$). However, at 100 mg/kg/day the differences from the controls in both sexes were < 5%. Since direct dosing did not affect weight gain from Day 7 at this dose level, the difference on Day 7 was considered treatment-related but not adverse.

Table 125
Group mean body weight of offspring prior to commencement of direct treatment

Treatment (mg/kg/day)	Sex	Group mean body weight (g) on lactation day:					
		1	4 (pre-cull)	4 (post-cull)	7	14	21
[a]Percent change compared to control group	Male (mean±SD)	7.0±0.7	10.0±1.2	10.1±1.2	16.7±1.9	35.2±3.1	55.5±4.5
100		6.8±0.4	9.6±0.9	9.6±0.8	16.1±1.5** (↓4)	34.2±3.1* (↓3)	55.7±5.1
250		6.6±0.7	9.1±1.3* (↓9)	9.2±1.2* (↓9)	15.3±2.4** (↓8)	31.6±4.9** (↓10)	51.1±7.7** (↓8)
500		6.7±0.6	9.2±1.2* (↓8)	9.3±1.1* (↓8)	15.5±1.5** (↓7)	28.9±3.7** (↓18)	48.4±5.4** (↓13)
0	Female (mean±SD)	6.7±0.6	9.6±1.2	9.5±1.3	15.8±1.8	33.6±3.2	53.3±4.5
100		6.5±0.4	9.0±0.8	9.0±0.8	15.1±1.3** (↓4)	32.6±3.3 (↓3)	52.8±5.0 (↓4)
250		6.3±0.6* (↓6) [a]	8.8±1.0* (↓3)	8.9±1.0 (↓6)	14.9±1.9** (↓6)	31.2±3.7** (↓7)	50.0±5.8 (↓4)
500		6.3±0.* (↓6)	8.7±1.1 (↓4)	8.7±1.2* (↓8)	14.7±1.8** (↓7)	27.3±4.3** (↓19)	46.9±6.0** (↓12)

* $p < 0.05$; ** $p < 0.01$. [a] Percent change compared to control group. Group mean data found in Tables 19-23 found on pages 118-124 of main study, MRID 47614917.

At 250 or 500 mg/kg/day, mean bodyweight gain of males and females was lower than the controls during the first week of direct treatment (Table 126). Following the cessation of treatment to Day 35 of age mean bodyweight gain of males and females in the 500 mg/kg/day group was marginally, but statistically significantly, lower than control. Thereafter, mean bodyweight gains were similar to the controls. At 100 mg/kg/day, mean bodyweight gain of offspring from Day 7 to day 63 was unaffected by treatment.

Table 126
Group mean body weight gain of offspring from commencement of treatment to day 63

Treatment (mg/kg/day)	Sex	Bodyweight on Day 7	Mean bodyweight gain (g) on days:			
			Days 7-13	Days 13-21	Days 21-35	Days 35-63
0	Male (mean±SD)	16.7±1.9	15.9±1.7	22.9±2.3	99±11	248±25
100		16.1±1.5** (↓4)[a]	15.6±2.0	24.0±2.9	96±8	246±20
250		15.3±2.4** (↓8)	13.7±2.6** (↓14)	22.0±2.3	96±9	244±23
500		15.5±1.5** (↓7)	10.8±2.5** (↓32)	22.1±2.5	91±13	241±28
0	Female (mean±SD)	15.8±1.8	15.3±2.0	22.2±2.7	79±8	114±16
100		15.1±1.3** (↓4)	15.0±2.4	22.6±3.0	77±8	118±14

250		14.9±1.9** (↓6)	13.9±1.9** (↓9)	21.3±3.0	77±8	114±17
500		14.7±1.8** (↓7)	10.2±2.7** (↓33)	22.0±2.7	73±7	116±14

**p < 0.01. [a] Percent change compared to control group. Individual data found in Table Appendixes 21 and 22 on pages 301-357 of main study, MRID 47614917.

During the offspring treatment period there was no effect on the incidence of deaths in the treated groups. There were 4, 4, and 3 male deaths in order of ascending dose level compared to 4 control male deaths. There were 1, 6 and 8 female deaths in order of ascending dose level compared to 5 control female deaths. In most instances for all treated groups, necropsy revealed evidence of dosing trauma. Therefore, offspring mortality in the treated groups was considered not to be related to the administration of penthiopyrad.

There was no effect of treatment at any dose level on the timing of the completion of balano-preputial separation of male offspring, or on the timing of the completion of vaginal opening in female offspring. Mean bodyweight at these times had no influence on occurrence of these landmarks.

Among surviving pups, yellow/brown/red staining of the perianal region with a low incidence of associated skin reddening/encrustations were evident among the majority of offspring receiving 500 mg/kg/day during the treatment period. Following the cessation of treatment there were no signs observed that were considered to be related to previous treatment with penthiopyrad.

Table 127
Offspring mortality prior to dosing postnatal days 7-21.

	Males			
	0 mg/kg/day	100 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Group Size	82	84	86	88
No. Deaths	4	4	4	3
Deaths due to dosing trauma	3	4	3	1
Deaths with no evidence of dosing trauma	1	0	1	2
	Females			
Group Size	84	75	87	88
No. Deaths	5	1	6	8
Deaths due to dosing trauma	0	1	4	3
Deaths with no evidence of dosing trauma	5	0	2	5

Data found on page 60 of main study, MRID 47614917.

Other than the perianal staining noted during routine observation, there were no treatment-related in-the-hand FOB observations at any of the observation intervals. Adverse treatment-related effects observed in the arena were confined to 5/22 offspring of each sex at 500 mg/kg/day that showed occasional whole body tremor (grade 1 - slight) on Day 21 of age, compared with control incidences of 0/21 and 1/21 for males and females, respectively (Table 128). The incidences of this observation at subsequent examinations were comparable in treated and control groups. There were no other effects of treatment on the performance of males or females in the arena that were considered to be of toxicological significance. On Day 21 of age, the observation of licking around the mouth was recorded for five males receiving 500 mg/kg/day and chewing mouth movements were seen in a few males in all treated groups, but not among controls. These observations were considered to be due to the anticipation of oral dosing and associated taste of the test compound, possibly resulting in increased salivation. The arena mean activity score for males in the 500 mg/kg/day group (19.2) was statistically higher ($p<0.05$) than the control value of 14.6 on Day 60 of age. However, there were no statistically significant differences in arena activity scores on any other test occasion and motor activity scores for the same animals on Day 59 of age were also unaffected by treatment.

Therefore, the differences in arena activity scores on Day 60 of age were attributed to natural variation. Observations on all other occasions were unaffected by treatment.

Table 128.
Selected FOB observations on offspring in the arena

Treatment (mg/kg/day)	Sex	Observation interval	Group value for:	
			No. with whole body tremor/no. tested	Mean activity score (sector entries)
0	Male	Day 4	0/21	2.0
100			0/20	1.9
250			0/22	1.4
500			0/22	1.4
0	Female		0/21	1.9
100			0/20	1.5
250			0/22	1.7
500			0/22	1.4
0	Male	Day 11	0/21	6.1
100			0/20	6.5
250			0/22	7.1
500			0/22	8.1
0	Female		0/21	8.3
100			0/20	7.9
250			0/22	9.4
500			0/22	7.7
0	Male	Day 21	0/21	7.4
100			1/20	7.3
250			2/22	6.3
500			5/22	8.7
0	Female		1/21	7.0
100			0/20	7.8
250			0/22	6.0
500			5/22	7.6
0	Male	Day 35	1/21	13.3
100			2/20	9.6
250			2/22	12.0
500			1/22	15.4
0	Female		0/21	13.3
100			0/20	12.4
250			1/22	11.9
500			0/22	17.3
0	Male	Day 45	1/21	14.4
100			0/20	11.7
250			0/22	14.4
500			0/22	14.5
0	Female		0/21	21.0
100			0/20	20.4
250			1/22	21.5
500			0/22	23.2
0	Male	Day 60	0/21	14.6±7.0
100			0/20	17.0±7.8
250			0/22	16.7±7.5
500			1/22	19.2±7.0*
0	Female		0/21	26.2±5.1
100			0/20	24.3±7.5

250			0/22	27.3±7.5
500			0/22	27.6±4.8

Group mean data for activity found in Tables 30-35 on pages 136-152 of main study, MRID 47614917.

A treatment-related increase in motor activity was observed at 500 mg/kg/day. At 500 mg/kg/day, motor activity was consistently greater than concurrent controls on all test days, ranging from 9-85% for rearing activity and 5-97% for cage floor activity. Although the increase was only significantly different from controls (α=0.05) in both sexes on day 17, this effect is considered adverse due to the consistent dose-response observed at 500 mg/kg/day. Increased activity was also noted in 100 and 250 mg/kg/day dose groups. However, at 100 and 250 mg/kg/day, the increase did not demonstrate a consistent dose-response relationship and was not significantly greater than controls, with one exception. On day 59, there was an increase in rearing activity in the 100 and 250 mg/kg/day groups compared to controls. However, this increase was only observed in females, the scores were within the HCD range, and there was not a concurrent increase in cage floor activity. Therefore, the increased motor activity observed at 100 and 250 mg/kg/day on day 59 is not considered adverse.

Details of motor activity by day:

> On Day 13 of age, there were some minor inter-group differences in mean high beam scores (rearing activity) and mean low beam scores (cage floor activity), the differences were small, did not show a dose-response relationship, and none were significantly different from control values, except the 500 mg/kg/day female 12-minute interval value for high beam counts.
>
> on day 17 of age, high beam and low beam scores (rearing and cage floor activity, respectively) for males at 500 or 250 mg/kg/day and females at 500 mg/kg/day were high relative to the controls during the latter part of the 1-hour recording period. The increased activity scores were greatest in males receiving 500 mg/kg/day, were statistically significant ($p<0.05$ or 0.01) for total scores and several of the 6-minute interval high and low beam scores, most of which were greater than the historical control data (HCD) range. The higher scores for females receiving 500 mg/kg/day and for males receiving 250 mg/kg/day were less marked, but total high beam score for males was above the HCD range and some of the 6-minute interval scores in both groups attained statistical significance. Activity scores for females receiving 250 mg/kg/day and for males and females receiving 100 mg/kg/day were unaffected by treatment.
>
> Motor activity scores were unaffected by treatment on day 22 of age. High beam and low beam scores for males receiving 500 mg/kg/day were high compared with controls for some of the 6-minute intervals but statistical significance ($p<0.05$) was attained in only two cases and the overall pattern of activity over the 1-hour recording period was considered to be essentially similar in all groups of males and females.
>
> High beam scores for females in all treated groups were high relative to the controls, for most of the 1-hour recording period on Day 59 of age, with some of the differences attaining statistical significance and with a few of the 6-minute interval scores (but not total scores) being above the HCD range. However, there was no evidence of a dose-relationship, all group mean total scores were within the HCD range and low beam scores for all treated groups were comparable to control scores. These differences were therefore not considered treatment-related. Group mean high and low beam scores for males were similar in all treated and Control groups.

Table 129
Group mean quantitative motor activity scores on offspring

Treatment (mg/kg/day)	Sex	Observation interval	Group mean total scores for:	
			High beam breaks (rearing activity) (mean±SD)	Low beam breaks (cage floor activity) (mean±SD)
0	Male	Day 13	14.8±22.4	623.0±445.9
100			14.1±22.7	566.5±458.9
250			26.5±37.8	489.4±280.0
500			23.3±44.2 (↑57)[a]	755.5±470.8 (↑21)
HCD[b] min-max (mean)			9.5-17.8[b] (13.8)	326.4-493.1 (406.2)
0	Female		25.9±28.0	570.1±427.5
100			21.1±36.5	684.7±355.9
250			16.8±33.3	470.7±291.9
500			38.1±42.7 (↑47)	717.3±429.9 (↑26)
HCD			6.5-25.8 (17.8)	173.4-633.1 (448.4)
0	Male	Day 17	119.6±137.6	684.4±485.8
100			113.7±120.8	925.2±888.1
250			203.8±156.3	1109.0±676.7
500			220.2±198.1* (↑85	1344.9±818.2** (↑97)
HCD			35.4-130.2 (72.0)	317.8-1165.3 (742.2)
0	Female		158.0±147.4	932.2±626.4
100			175.8±178.2	906.2±714.6
250			185.3±162.4	971.4±653.7
500			225.8±194.9 (↑43)	1332.3±809.0 (↑43)
HCD			105.8-243.3 (151.1)	830.7-1421.3 (1048.5)
0	Male	Day 22	183.1±104.1	472.5±250.9
100			196.4±133.0	456.8±269.4
250			177.5±74.0	502.8±208.8
500			236.0±162.4 (↑29)	618.7±382.3 (↑31)
HCD			102.7-259.4 (155.8)	326.9-729.5 (468.0)
0	Female		152.9±110.0	386.5±204.9
100			183.3±111.1	493.8±241.7
250			160.4±98.7	482.1±235.3
500			194.1±172.5 (↑27)	527.4±356.4 (↑36)
HCD			97.8-182.5 (142.9)	379.7-492.9 (447.0)
0	Male	Day 59	492.6±149.4	1205.2±270.2
100			428.2±128.8	1240.6±270.1
250			484.2±180.9	1230.6±311.9
500			534.7±174.6 (↑9)	1270.1±306.3 (↑5)
HCD			364.5-649.7 (461.5)	1150.3-1652.8 (1365.7)
0	Female		550.9±171.2	1270.8±363.0
100			729.3±262.8*	1326.7±436.8
250			806.5±334.8**	1514.1±331.6
500			767.8±319.1** (↑39)	1452.8±382.1 (↑14)
HCD			391.6 - 813.5 (589.9)	1135.9 - 1718.8 (1392.9)

* $p < 0.05$; ** $p < 0.01$; [a]percent increase in activity compared to concurrent controls (calculated by reviewer); [b] HCD derived from 4 studies from February 2004 - September 2007. Route of administration (gavage vs. Dietary not specified for Historical Control Data. Route of administration (gavage vs. Dietary) was not specified for Historical Control Data. ; [b] range of total 1-hour scores,. Group mean data found on pages 153-160 of main study, MRID 47614917. HCD data found on pages 807-814, MRID 47614917

Pre-pulse inhibition of the auditory startle response on day 23/24 and day 61/62 was unaffected by treatment at all dose levels. The group mean percent inhibition values in both sexes at all dose levels were comparable to, and not significantly different ($p > 0.05$) from, control values on both measurement occasions. Additionally, latency to peak stimulus with and without pre-pulse were comparable in all treated and control groups on both testing occasions. However, peak amplitudes for both sexes at 500 mg/kg/day were low compared with the controls on Day 23/24 of age, with group mean values in response to the stimulus both with and without the pre-pulse being statistically significant ($p < 0.05$) for all values except females without the pre-pulse. These low amplitude values were considered to be due to the low bodyweights in this group, since the percent inhibition with pre-pulse was unaffected and because further statistical analysis of peak amplitudes, using bodyweight as covariate, showed no statistically significant differences ($p > 0.05$) between the groups at Day 23/24 of age.

Peak amplitude values both with and without a pre-pulse for females in the 500 mg/kg/day group were significantly lower than the controls ($p < 0.05$) at Day 61/62 of age as well. However, the group mean percent inhibition was not significantly different from control values. There were no treatment-related differences in bodyweights at this age, so the low amplitude values could not be associated with low bodyweights.

Table 130
Summary of auditory startle response pre-pulse inhibition

Treatment (mg/kg/day)	Age (days) and sex	Group mean value for:				
		Latency to peak (ms) (mean±SD)		Peak amplitude (g) (mean±SD)		
		- pre-pulse	+ pre-pulse	- pre-pulse	+ pre-pulse	% inhibition
0	23/24 Males	14.2±4.4	14.2±1.3	153.0±32.2	122.9±25.0	19.2±7.0
100		13.3±1.0	13.8±1.7	160.2±27.5	129.4±25.1	19.2±7.7
250		13.0±0.9	14.0±1.6	148.6±29.3	116.4±22.7	21.1±7.7
500		14.1±2.2	14.0±1.5	129.2±31.3*	103.2±24.3*	19.3±10.6
0	23/24 Females	13.6±1.9	13.8±1.3	147.4±26.8	116.4±17.3	20.1±9.2
100		13.6±1.7	13.8±1.5	146.0±27.1	119.0±24.9	18.7±5.7
250		13.3±1.7	13.9±1.6	135.3±29.3	107.0±21.0	19.9±8.6
500		14.1±2.3	14.5±1.5	130.1±28.5	99.6±20.3*	22.7±8.3
0	61/62 Males	15.3±3.2	16.5±3.3	754.8±162.0	572.5±120.4	23.4±8.8
100		16.3±4.7	16.8±3.4	804.8±234.5	591.3±120.4	24.9±8.0
250		15.0±3.9	16.9±4.9	757.9±190.2	549.1±93.7	25.2±12.8
500		14.4±2.4	17.5±5.2	774.3±226.1	549.7±147.7	27.1±13.2
0	61/62 Females	16.4±4.3	18.6±4.8	535.5±179.8	370.9±84.1	28.4±11.2
100		16.7±4.9	17.0±3.5	521.0±168.8	394.1±99.3	22.2±11.8
250		18.4±6.4	19.7±6.8	461.5±107.2	334.0±60.9	26.4±9.7
500		17.9±5.3	19.9±5.8	428.5±*128.3	316.5±61.3*	23.7±11.4

* $p < 0.05$, Group mean data found in Tables 40 and 41 on pages 161-164, MRID 47614917.

Learning and memory, as assessed by performance in the Morris water maze from Day 23/24 and from Day 61/62 of age were unaffected by treatment in both sexes at all dose levels. All treated and control groups of both sexes showed clear evidence of learning (acquisition) and subsequent recall from memory during the 4-day testing regimen. There was a progressive decrease in the mean trial times and the numbers of pool sector entries and failed trials during the testing period. Thus, the progressive improvement in the performance of the animals is clear evidence of learning (acquisition) and subsequent recall from memory from day to day. The improvement in performance from Day 1 to Day 2 of testing was considered to reflect short-term memory on Day 1 being consolidated into acquisition learning evident on Day 2, while the overall improvement in performance over the 4 days of testing was considered to be a measure of long-term memory. All group mean values for trial time, and numbers of sector entries and failed trials in the treated groups were not significantly different ($p > 0.05$) from control values, with the exception of trial time and sector entry values for males at 100 mg/kg/day on Day 23/24 of age which were significantly lower than Control values ($p < 0.05$) on Day 3 of the test. These differences were considered to be fortuitous in view of the direction of change relative to Controls (improved performance) and the absence of effects at higher dose levels.

At necropsy, the nature, incidence and group distribution of all macroscopic findings in perfused offspring at day 21 or day 66 of age did not indicate an effect of treatment at any dose level. Similarly, the nature, incidence and group distribution of all macroscopic findings in unselected offspring on Day 28 of age and non-perfused offspring on Day 66 of age did not indicate an effect of treatment at any dose level.

There was no effect of treatment on absolute and adjusted brain weights in day 21 and day 66 perfused offspring or in unperfused offspring at day 66. None of the group mean values in the treated groups were significantly different ($p > 0.05$) from control values.

There was no effect of treatment at 500 mg/kg/day on the four brain morphometry measurements in perfused offspring on day 21 or day 66 of age. None of the group mean values in the treated groups was significantly different ($p > 0.05$) from control values.

Although brain length and width measurements were not recorded in day 21 perfused offspring, in error, brain weight and morphometry measurements in these offspring did not indicate an effect of treatment at any dose level. Therefore, it is also considered that brain length and width at day 21 would not have been affected by treatment at any dose level. In day 66 offspring, males at 500 or 250 mg/kg/day, had mean brain lengths which were marginally but statistically significantly lower than control, but the difference in brain length amounted to less than 2% (Table 131). In the absence of an effect on mean brain weight and on the brain morphometry measurements of 4 specific regions, these minimal differences from control were considered unrelated to treatment. The brain dimensions in males at 100 mg/kg/day, and in all treated female groups were comparable to control values.

Table 131.
Summary of brain dimensions in day 66 perfused offspring

Treatment (mg/kg/day)	**Group mean value (mm) in:**			
	Males(mean±SD)		**Females** (mean±SD)	
	Length	**Width**	**Length**	**Width**
0	21.3±0.4	15.5±0.4	20.5±0.3	15.2±0.4
100	21.1±0.4	15.5±0.3	20.5±0.4	15.1±0.3
250	20.9±0.4*	15.3±0.4	20.4±0.7	15.2±0.3
500	20.9±0.3*	15.3±0.2	20.4±0.4	15.0±0.2

* $p < 0.05$. Group mean data found in Table 54 on page 183 of main study, MRID 47614917 .

There were no treatment-related changes in the tissues of the central and peripheral nervous systems, eyes and skeletal muscle presented for pathological examination in day 21 or day 66 perfused offspring in the 500 mg/kg/day group. Changes in other tissues were of a type and severity commonly seen in rats of this age at this laboratory. In day 66 offspring, there were variable incidences of minimal or slight degenerative changes in the peripheral nerves. These changes occurred at similar incidences in control and high dose animals and were considered to be unrelated to treatment (Table 132). Other changes were of a type and severity commonly seen in rats of this age at this laboratory.

Table 132.
Summary incidence of histopathological findings in peripheral nerves of offspring

Tissue - lesion	**Incidence / no. examined in:**			
	Males treated at (mg/kg/day):		**Females treated at (mg/kg/day):**	
	0	**500**	**0**	**500**
Sciatic nerve (thigh) - degenerate fibres	4 / 9	4 / 10	3 / 10	5 / 10
Sciatic nerve (notch) - degenerate fibres	7 / 9	7 / 10	3 / 10	4 / 10
Tibial nerve (knee) - degenerate fibres	3 / 9	3 / 10	2 / 10	2 / 10
Tibial nerve (calf) - degenerate fibres	4 / 9	4 / 10	2 / 10	4 / 10

Data found in Table 48 on pages 173-174 in the main study, MRID 47614917.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable (Acceptable/non-guideline)

This study is fully compliant with OECD 471(1997)

C. CONCLUSIONS:

A maternal no-observed-adverse-effect-level (NOAEL) was established as 500 mg/kg/day. The lowest-observed adverse-effect-level (LOAEL) was not determined based on the absence of maternal adverse effects and changes in behaviour at the highest dose level employed.

A NOAEL for offspring toxicity was established as 100 mg/kg/day based on slightly lower Day 1 bodyweights and subsequent bodyweight gain, and increased motor activity observed at the LOAEL of 250mg/kg/day. At 500 mg/kg/day, increased motor activity and whole body tremors (on Day 21) were seen.

IIA 5.7.6 Summary neurotoxicity

Acute and 13-week oral neurotoxicity studies and a developmental neurotoxicity study have been performed in the rat according to OPPTS and OECD guidelines. Acute and 28-day delayed neurotoxicity studies have not been performed because penthiopyrad has no chemical similarities to structures known or implicated in producing delayed neurotoxicity.

Table 133
Summary of neurotoxicity studies performed on penthiopyrad

Study / dose levels	NO(A)EL (mg/kg/day)	LO(A)EL (mg/kg/day)	Reference
Acute oral neurotoxicity in the rat; 0 - 125 - 500 - 2000 mg/kg	125	500[a]	Chapman (2008b) IIA 5.7.1/02
13-week dietary neurotoxicity in the rat; 0 - 10 - 40 - 160 - 640 mg/kg/day	640 160[b]	640	Groom (2008b) IIA 5.7.4/01
Oral developmental neurotoxicity in the rat; 0 - 100 - 250 - 500 mg/kg/day	100[c]	250	Stannard (2009b) IIA 5.7.5/02

[a] based on transient functional alterations

[b] NOAEL for all effects was 160 mg/kg/day based on reduced body weight gain at LOAEL of 640 mg/kg/day

[c] based on increased notor activity and decreased pup weight

Administration of a single oral dose of 500 or 2000 mg/kg penthiopyrad produced a range of functional effects at the time of peak effect on the day of treatment. The effects included abnormal posture/gait, reduced motor activity and reduced body temperature. Whole body tremor also occurred in some animals treated at 2000 mg/kg/day. None of the functional neurobehavioural effects persisted beyond the day of treatment. There were no effects at any dose level on brain weight and dimensions, and no neurohistopathological alterations in the central and peripheral nervous systems at 2000 mg/kg. The acute NOAEL was determined as 125 mg/kg based on the occurrence of transient functional effects.

In contrast to the acute study, penthiopyrad did not elicit either functional or histopathological evidence of neurotoxicity following 13-weeks treatment at dose levels up to the maximum tolerated dose of 640 mg/kg/day. The NOAEL in the study was determined as 160 mg/kg/day based on decreased body weight gain in males at 640 mg/kg/day.

In the developmental neurotoxicity study, dose levels up to 500 mg/kg/day were well-tolerated by the maternal animals. Transient neurobehavioural effects were observed in offspring at dose levels of 250 or 500 mg/kg/day. The effects occurred at one testing interval only and comprised an increased incidence of slight whole body tremors at

500 mg/kg/day and increased motor activity at 250 or 500 mg/kg/day. Quantitative assessments of sensory function and learning and memory in offspring were unaffected by treatment at all dose levels and there were no effects on brain weight, dimensions and morphometry of specific regions. No neurohistopathological alterations were evident in offspring at 500 mg/kg/day. Consequently, the NOAEL in offspring was determined as 100 mg/kg/day, based on increased motor activity and decreased body weight at 250 mg/kg/day.